

Electrochemical detection of hybridized DNA using reduction of methylene blue

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Abstract - One of the important roles of a DNA sensor is the capability of detecting genetic diseases or mutations by analyzing DNA sequence. The electrochemical detection method can be simpler and cheaper than other methods available and hence this method was investigated in this paper. For a successful electrochemical detection, several aspects should be considered including chemical treatment of electrode surface, DNA immobilization on electrode, hybridization, choice of an intercalator to be bound to double strand DNA selectively, and an equipment for detecting and analyzing the output signal. The intercalator bound to double strand DNA results in an electrical current. With the electrochemical detection method, double strand DNA was distinguished from single strand DNA or bare gold electrode by measuring reduction current of intercalator. Also, it was found that the reduction current of intercalator is proportional to the concentration of target DNA to be hybridized with probe DNA. Therefore, it is possible to realize a simple and cheap DNA sensor using the electrochemical detection method for DNA sequencing.

Keywords - DNA, electrochemical detection, intercalator

I. INTRODUCTION

With the progress of biotechnology, the efforts of detecting genetic diseases and mutations for improving functions of living organism through the analysis of DNA sequence have increased recently [1]. There are several methods presented by other researchers for the analysis of the DNA sequence, including mass sensitive detection method [2,3], optical method [4,5], and electrochemical method [6-9].

Among these methods, the electrochemical detection method has several advantages compared to other methods. First, the electrochemical detection system can be cheap and small because it requires three-electrodes, a signal generator, and a signal processing chip. This system can be much cheaper than other detection systems because it doesn't require complicated parts. Second, the process of the electrochemical detection is fairly simple, and the acquisition time of results is short. Last, the outputs from the electrochemical measurement are electrical signals, which easily can be applied to other electronic system.

For a successful electrochemical measurement, several aspects should be considered such as choice of electrode material, chemical treatment (or modification) of the electrode surface, immobilization condition of probe DNA on the modified electrodes, target DNA hybridization condition, and choice of an intercalator to be bound to double strand DNA (dsDNA) selectively. The intercalator bound to the dsDNA is believed to cause an electric current by its reduction. In this research, a commercially available electrochemical analysis system was used to make redox

condition for experiments and to measure the reduction current due to the intercalator.

This paper presents the process of the electrochemical detection method for a specific DNA sequence and the experimental results using Au electrodes with 2-mercaptoethanol as electrode surface treatment material and methylene blue as an intercalator. The result shows a distinct peak current difference between dsDNA and a single strand DNA (ssDNA) from a cyclic voltammetry. The dependence of reduction peak current on the methylene blue concentration was also observed when the target DNA was hybridized with the probe DNA.

II. EXPERIMENTAL PROCEDURE

1) Preparation of Electrodes:

Zhao et al. reported the hydroxyl-terminated self-assembled monolayer (SAM) is a good substrate for the covalent immobilization of dsDNA on gold surface [10]. In this experiment, Au was chosen as electrode material, and a gold electrode with 2mm² in area was purchased from Bioanalytical Systems (BAS). It was soaked in boiling 2 M NaOH for 5 minutes, concentrated nitric acid for 5 minutes, and piranha solution for 3 minutes in order and washed by vortexing for 3 minutes twice. After cleaning, the electrode was voltammetrically cycled and characterized from 0.1 V to 1.5 V vs. Ag/AgCl in 0.1 M H₂SO₄ solution until a stable cyclic voltammogram was obtained using a BAS Model CV-50W electrochemical analyzer. The electrode was then soaked in 1 mM 2-mercaptoethanol (2-ME) solution for 2 hours to make 2-ME layer onto the electrode surface. Thus a 2-ME SAM modified gold electrode was obtained (2ME/Au).

2) Probe DNA Immobilization:

The 2ME/Au was reacted with 1 nmol probe DNA (5'-TCT TTT GGC GGT ATG CAC TT-3') in the presence of 10 µg 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDAC) in 1 µl 40 mmole 2-[N-morpholino]ethanesulfonic acid (MES) buffer (pH 4.5) for 24 hours at room temperature. As a result, a 2-ME/Au immobilized with single strand DNA is obtained (ssDNA-2ME/Au).

3) Hybridization:

The ssDNA-2ME/Au was reacted with 1 nmol target DNA (5'-AA GTG CAT ACC GCC AAA AGA-3') in 30 µl hybridization solution containing 2.7 µg salmon sperm DNA, 175 µg MES acid, 481 µg MES potassium salt, 26.6 µmol NaCl, 0.6 µmol ethylene diaminetetraacetate (EDTA) and 0.01% Tween 20 in 30 µL hybridization solution for 24 hours

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at 37°C. In order to eliminate nonspecifically bound DNAs and contaminants after hybridization, the electrode was washed in the washing solution containing 59 mg MES acid, 161 mg MES sodium salt, 0.26 mmol NaCl and 0.01% Tween 20 for 15 minutes at 37°C three times. As a result, an ssDNA/Au hybridized with target DNA was obtained (dsDNA-2ME/Au).

4) Intercalation and Electrochemical Measurements:

2ME/Au, ssDNA-2ME/Au, dsDNA-2ME/Au made by above steps were soaked in the presence of 5 mM phosphate salt buffer (pH 7.0), 50 mM NaCl, 0.2 mM potassium ferricyanide ($[\text{Fe}(\text{CN})_6]^{3-}$), 0.5 μM methylene blue in electrolyte for 10 minutes at room temperature under a dark condition, and the cyclic voltammetry (CV) and the chronocoulometry (CC) were carried out at room temperature with a 2ME/Au or ssDNA-2ME/Au or dsDNA-2ME/Au as a working electrode, a Ag/AgCl reference electrode (BAS Model MW-4130) and Pt counter electrode (BAS Model MF-2052). Then a reduction current of methylene blue was measured by 1 cycle CV with -100 mV/sec scan rate and CC at -220 mV. Also, to examine the dependence of the concentration of target DNA on the output current, each ssDNA-2ME/Au was hybridized with 0, 0.1, 0.5, 1 nmol/30 μL target DNA as procedure 3), and then CV and CC were carried out. The measured data was stored to a computer connected with electrochemical analyzer.

III. RESULTS & DISCUSSION

For the electrochemical measurement, chemical treatment of the electrode surface to immobilize probe DNA on the electrode must be considered. Therefore, Au was used as electrode material, and 2-ME layer was formed onto the Au electrode. After immobilization of probe DNA on 2ME/Au, ssDNA-2ME/Au was hybridized with a target DNA, and then dsDNA-2ME/Au was made (Fig. 1).

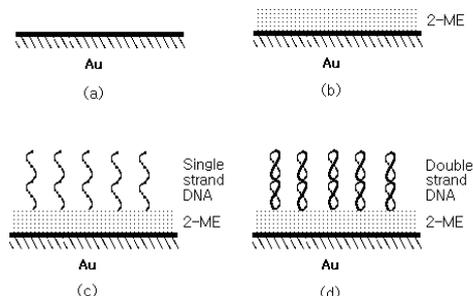


Fig. 1. (a) Bare Au, (b) 2ME/Au, (c) ssDNA-2ME/Au, (d) dsDNA-2ME/Au made at each process of the experimental procedure.

These electrodes were soaked in the presence of potassium ferricyanide, methylene blue in electrolyte for 10 minutes, and then methylene blue was bound in base pairs of double strand DNA selectively. Therefore, a larger reduction current

with methylene blue was produced in dsDNA-2ME/Au than both 2ME/Au and ssDNA-2ME/Au when the electrochemical measurement was carried out. There is a schematic representation of redox by the electrochemical method on dsDNA-2ME/Au in Fig. 2. Electrons flow from the electrode to intercalated methylene blue in base pairs of double strand DNA and then are accepted by $[\text{Fe}(\text{CN})_6]^{3-}$ in solution. Chemically oxidized methylene blue is again available for electrochemical reduction. This process can be repeated until the electrode is no longer at a potential to reduce methylene blue or all $[\text{Fe}(\text{CN})_6]^{3-}$ in the solution.

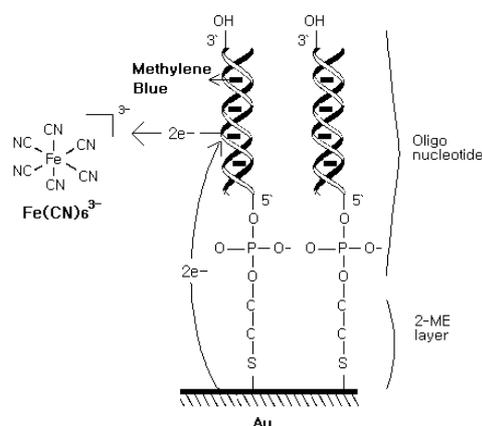


Fig. 2. Electrons ($2e^-$) flow from the electrode to intercalated methylene blue in base pairs of double strand DNA and then are accepted by $[\text{Fe}(\text{CN})_6]^{3-}$ in electrolyte solution.

The Au electrodes were measured in three electrodes, one-compartment cell with an Ag/AgCl reference electrode and a Pt counter electrode. This measurement system is shown in Fig. 3.

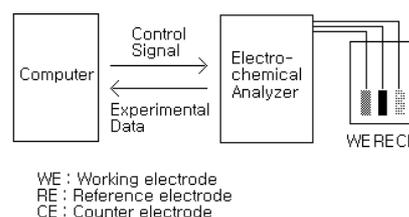


Fig. 3. Electrochemical measurement system

The maximum reduction current of methylene blue occurred at -220 mV with CV, and the results from six times measurement using different Au electrodes and reagents was shown in Fig. 4. Rectangles and lines represent the average and the standard deviation of measured values from each electrode, respectively. The graph of Fig. 4 shows that the average values of the peak reduction currents of methylene blue are about 17 nA (2ME/Au), 22 nA (ssDNA-2ME/Au), 115 nA (dsDNA-2ME/Au). They prove that methylene blue binds more to dsDNA than ssDNA. Therefore, it is possible

that a fabrication of DNA sensor that can distinguish dsDNA from ssDNA in just a few seconds by the electrochemical measurement.

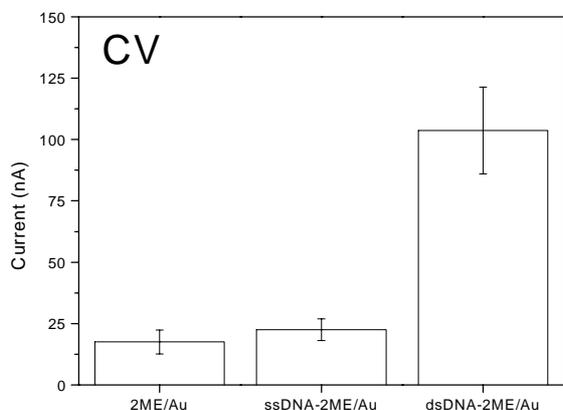


Fig. 4. The maximum values of current caused by reduction of methylene blue in 2ME/Au, ssDNA-2ME/Au, and dsDNA-2ME/Au at -220 mV. The result was calculated after six times measurement using different Au electrodes and reagents by CV with -100 mV/sec scan rate from -100 mV to -400 mV. Rectangles and lines represent the averages and the standard deviations of measured value respectively.

The possibility of detection of dsDNA by CC as well as CV was proved, and the result from six times measurement using different Au electrodes and reagents of CC was shown in Fig. 5. Rectangles and lines represent the averages and the standard deviations of measured values from each electrode, respectively. Figure 5 shows that the average values of charge of methylene blue are $1.7 \mu\text{C}$ (2ME/Au), $2.5 \mu\text{C}$ (ssDNA-2ME/Au), $2.9 \mu\text{C}$ (dsDNA-2ME/Au) at 5 seconds and $2.9 \mu\text{C}$ (2ME/Au), $4.1 \mu\text{C}$ (ssDNA-2ME/Au), $5.0 \mu\text{C}$ (dsDNA-2ME/Au) at 10 seconds. This result supports the former result obtained from the CV measurements.

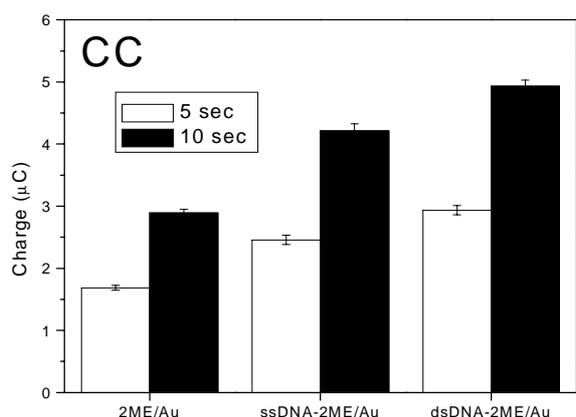


Fig. 5. The average values of charge caused by methylene blue in 2ME/Au, ssDNA-2ME/Au, dsDNA-2ME/Au. The results were calculated after six times measurement using different Au electrodes and reagents by CC for 5 seconds (white rectangle) and 10 seconds (black rectangle) at -220 mV. The lines represent the standard deviations of measured value.

Next, in order to investigate the dependence of the concentration of target DNA for hybridizing with probe DNA on the output current, ssDNA-2ME/Au were hybridized at various concentrated target DNA. Identically with the above, CV and CC were carried out with each electrode, and the results from the six times measurement using different Au electrodes and reagents was shown in Fig. 6 and 7. Rectangular spots and vertical lines represent the averages and the standard deviations of measured values from each electrode. From these graph, the approximate results were shown in table 1. The results shows that the reduction current of methylene blue is proportional to the concentration of target DNA for hybridizing with probe DNA. Therefore, just by measuring output current from the electrode, quantitative analysis of double strand DNA is possible.

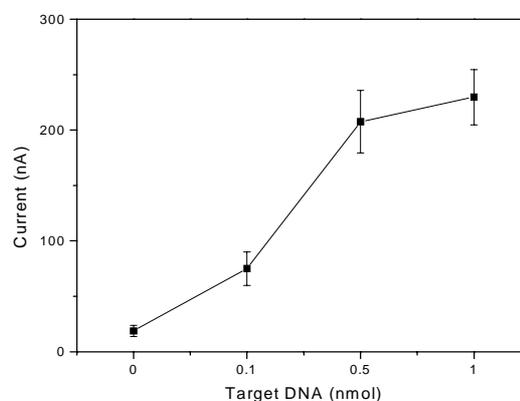


Fig. 6. The maximum values of current caused by reduction of methylene blue at -220 mV vs. the concentrations of target DNA. The result was calculated after five times measurement using different Au electrodes and reagents by cyclic voltammetry with -100 mV/sec scan rate from -100 mV to -400 mV. The rectangular black spots and the vertical lines represent the averages and the standard deviations of measured value respectively.

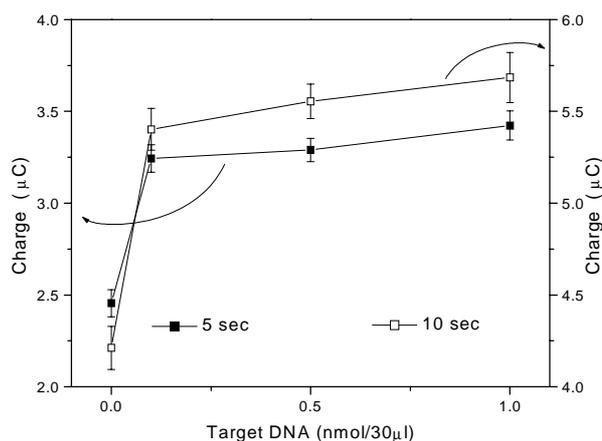


Fig. 7. The values of charge caused by reduction of methylene blue at -220 mV vs. the concentrations of target DNA. The results were calculated after five times measurement using different Au electrodes and reagents by chronocoulmetry for 5 seconds (rectangular black spots) and 10 seconds (rectangular white spots) at -220 mV. The vertical lines represent the standard deviations of measured value.

TABLE I
THE REDUCTION CURRENT AND CHARGE OF METHYLENE BLUE VS. TARGET DNA CONCENTRATION.

Concentration (nmol/30 μ l)	Cyclic Voltammetry (nA)	Chronocoulometry (μ C)	
		5 sec.	10 sec.
0.0	23 \pm 4	2.49 \pm 0.10	4.24 \pm 0.14
0.1	78 \pm 16	3.25 \pm 0.08	5.49 \pm 0.14
0.5	210 \pm 30	3.26 \pm 0.08	5.60 \pm 0.14
1.0	225 \pm 26	3.30 \pm 0.10	5.70 \pm 0.16

IV. CONCLUSIONS

The electrochemical measurement has a capability to distinguish dsDNA from ssDNA in just a few seconds, with either cyclic voltammetry or chronocoulometry. Even quantitative analysis of double strand DNA is possible just by measuring output current from the electrode. In order to take advantages of this simple electrochemical detection method, a small and cheap electronic control system would be required for increasing the minute current from DNA modified electrode. It will be possible that a fabrication of micro DNA sensor that have good yield, if microelectrode can be made by MEMS technology. It won't be far away to see that restoration and improvement of living organism's function through detecting of genetic disease and mutations by electrochemical measurement are realized.

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REFERENCES

- [1] Susan R. Barnum, *Biotechnology*, Wadsworth Publishing Company, 1998.
- [2] Bardea, A., Dagan, A., Ben-Dov, I., Amit, B. and Willner, I., "Amplified microgravimetric quartz crystal-microbalance analyses of oligonucleotide complexes: a route to a Tay-Sachs biosensor device," *Chemical Communications*, vol. 7, pp. 839-840, 1998.
- [3] J. Fritz, M. K. Baller, H. P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H. -J. Guntherodt, Ch. Gerber, J. K. Gimzewski, "Translating Biomolecular Recognition into Nanomechanics," *Science*, vol. 288, pp. 316-318, 2000.
- [4] Storhoff, J., Elghanian, R., Mucic, C., Mirkin, C. and Letsinger, R. "One-pot colorimetric differentiation of polynucleotides with single base imperfections using gold nanoparticle probes," *Journal of American Chemical Society*, vol. 120, pp. 1959-1964, 1998.
- [5] Bernard Juskowiak, Mitsuyoshi Ohba, Masao Sato, Shigeori Takenaka, Makoto Takagi, and Hiroki Kondo, "Photoisomerizable DNA Ligands. Spectral and Electrochemical Properties and Base-Pair Selectivity of Binding of Bis[2-(1-alkylpyridinium-4-yl)vinyl]benzene Dyes," *Bulletin of Chemical Society of Japan*, vol. 72, pp. 265-277, 1999.
- [6] Koji Hashimoto, Keiko Ito, and Yoshio Ishimori, "Novel DNA sensor for electrochemical gene detection," *Analytica Chimica Acta*, vol. 286, pp. 219-224, 1994.
- [7] Shigeori Takenaka, Yoshihiro Uto, Hideki Saita, Makoto Yokoyama, Hiroki Kondo and W. David Wilson, "Electrochemically active threading intercalator with high double stranded DNA selectivity," *Chemical Communications*, pp. 1111-1112, 1998.
- [8] Koji Hashimoto, Keiko Ito and Yoshio Ishimori, "Microfabricated disposable DNA sensor for detection of hepatitis B virus DNA," *Sensors and Actuators B*, vol. 46, pp. 220-225, 1998.
- [9] Adam B. Steel, Tonya M. Herne, and Michael j. Tarlov, "Electrochemical quantitation of DNA immobilized on gold," *Analytical Chemistry*, vol. 70, pp. 4679-4677, 1998.
- [10] Yuan-Di Zhao, Dai-Wen Pang, Shen Hu, Zong-Li Wang, Jie-Ke Cheng, and Hong-Ping Dai, "DNA-modified electrodes; part4: optimization of covalent immobilization of DNA on self-assembled monolayers," *Talanta*, vol. 49, pp. 751-756, 1999.